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Differential transcriptional activity of plant-pathogenic begomoviruses in their whitefly vector (*Bemisia tabaci*, Gennadius: Hemiptera Aleyrodidae)

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Plant-pathogenic begomoviruses have a complex association with their whitefly vector and aspects concerning virus genetic activity (genome replication and gene transcription) within the insect remain highly controversial. Virus transcript abundance was assessed by quantifying selected gene transcripts of *Tomato mottle virus* (ToMoV, a New World bipartite begomovirus) and *Tomato yellow leaf curl virus* (TYLCV, an Old World monopartite begomovirus) in whiteflies (*Bemisia tabaci* biotype B) after feeding on virus-infected tomato plants and after subsequent transfer to cotton, a plant that is immune to the selected begomoviruses. Real-time RT-PCR was performed using specific primers for three ToMoV genes (AV1, BC1 and BV1) and three TYLCV genes (V1, V2 and C3). The ToMoV gene transcripts rapidly became undetectable in whiteflies following transfer from tomato to cotton, probably because degradation was not accompanied by new synthesis. On the other hand, TYLCV transcripts increased after transfer of whiteflies to cotton, indicating active TYLCV transcription. Interestingly, the difference observed in ToMoV and TYLCV transcripts in the vector parallel observations on the different biological effects of these viruses on whiteflies, i.e. TYLCV, but not ToMoV, reduces whitefly fitness.

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INTRODUCTION

Many plant-pathogenic viruses are vectored by insects and the interactions between these viruses and their vectors range from the insect functioning as a casual carrier (minimal vector–insect interactions) to intimate molecular interactions, including circulative transmission (movement of the virus from the gut into the haemolymph and then back to the mouthparts, typically through the salivary gland) and propagative transmission (virus transcription and replication within the vector) (Nault, 1997). Replication within insect vectors is rarer for plant viruses than for mammalian insect-vectored viruses. To date, the only plant viruses proven to replicate in their vector are RNA viruses belonging to or related to the families *Rhabdoviridae*, *Reoviridae* and *Bunyaviridae* and at least one genus that is not associated with a family (*Marafivirus*). These families contain both plant and vertebrate viruses (van Regenmortel *et al.*, 2000).

Recent research, including the effect of virus on insect fecundity, apparent sexual transmission of virus in the insect and non-quantitative detection of virus coat protein and genome, has lead to the speculation that *Tomato yellow leaf curl virus* (TYLCV), a ssDNA plant virus that induces severe symptoms in tomato, may replicate in its whitefly vector (Czosnek *et al.*, 2001). However, this idea is controversial due to a lack of definitive proof and some conflicting data: Ghanim *et al.* (1998) versus Bosco *et al.* (2004). Definitive determination of the genetic activity of TYLCV awaits quantitative detection of *de novo* synthesized transcripts and viral genomes. Also, analysis of the genetic activity of other begomoviruses compared with that of TYLCV in the insect vector will provide information on the frequency of occurrence of virus genetic activity in the insect among this group of plant viruses.

In this report, work was performed to compare the transcriptional activity in *Bemisia tabaci* (Gennadius) biotype B of two different begomoviruses that represent two major subgroups. *Tomato mottle virus* (ToMoV) and TYLCV are two whitefly-transmitted ssDNA circular genome plant-pathogenic viruses that belong to the genus *Begomovirus* within the family *Geminiviridae* (Rybicki, 1994; Padidam *et al.*, 1995; Stanley *et al.*, 2004). ToMoV is a New World

A figure showing begomovirus detection in plants and vectors by real-time RT-PCR is available as Supplementary material in JGV Online.

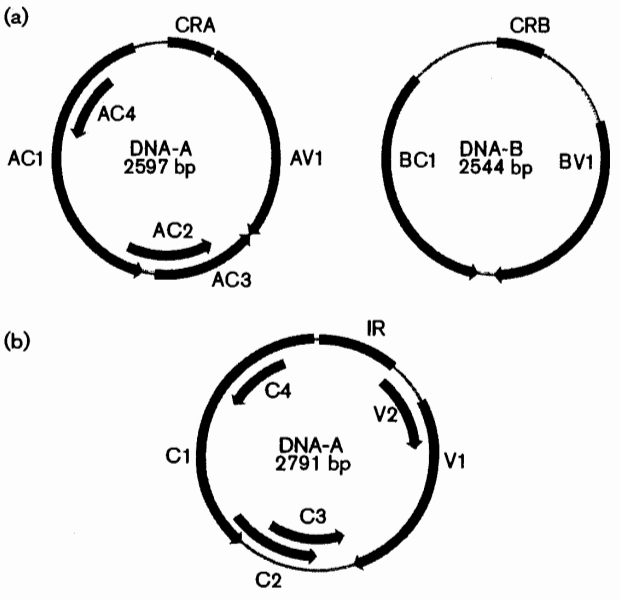


Fig. 1. Begomovirus genome organization. (a) Genome organization of *Tomato mottle virus*. (b) Genome organization of *Tomato yellow leaf curl virus*.

bipartite begomovirus (Fig. 1a), whereas TYLCV is a monopartite Old World begomovirus (Fig. 1b). These viruses have emerged as agricultural problems, with TYLCV being a serious threat to worldwide crop production (Polston & Anderson, 1997; Moffat, 1999).

Although both begomoviruses are transmitted by whiteflies in a persistent circulative manner (Hunter *et al.*, 1998; Rosell *et al.*, 1999; Ghanim *et al.*, 2001), differences in the association of ToMoV and TYLCV with whiteflies are apparent. TYLCV reduces the fecundity of whiteflies (Rubinstein & Czosnek, 1997) and detection of its genome and coat protein has been interpreted as evidence for possible replication of this virus in whiteflies (Czosnek *et al.*, 2001). Although evidence for the transovarial transmission of geminivirus DNA has been reported (Ghanim *et al.*, 1998; Bosco *et al.*, 2004), transovarial transmission of infectivity, claimed in the former article, was not confirmed in the latter, leaving the matter still controversial. In contrast, ToMoV-viruliferous whiteflies displayed higher fecundity on healthy tomato than non-viruliferous whiteflies (McKenzie, 2002; McKenzie *et al.*, 2002) and transovarial transmission and accumulation of viral proteins have not been reported. Whether these differences are due to differences in virus replication or other interactions between the insect and the virus remains to be determined. Presented here are the results of research comparing the transcriptional activity of ToMoV and TYLCV in their vector, *B. tabaci* biotype B.

METHODS

Source and maintenance of insects, plants and virus. Adult male and female whiteflies, *B. tabaci* (Gennadius) biotype B, were obtained from laboratory colonies maintained by the US Horticultural Research Laboratory, Fort Pierce, FL, USA, and originally provided by L. Osborne, University of Florida, Apopka, FL, USA. Colonies have been maintained on dwarf cherry tomato (*Lycopersicon esculentum* Mill. cv. Florida Lanai) since 1996 by serial transfers. Whitefly biotyping was based on PCR analysis using primers developed by De Barro & Driver (1997). Non-viruliferous and viruliferous whitefly colonies were housed separately in screened Plexiglass cages located in separate walk-in Environmental Growth Chambers at 25 ± 1 °C under a 16:8 light/dark photoperiod and a mean light intensity of 700 μE PAR at the top of the plant canopy.

Cotton plants (*Gossypium hirsutum* L. cv. Delta Pine 5415) were obtained from seed sown directly into ~15-cm pots. Seeds were allowed to germinate, thinned to six plants per pot and fertilized weekly with 20-10-20 Peters Professional Plant Starter Product (Scotts-Sierra Horticultural Products). Plants with three to four fully expanded leaves were used for whitefly infestations.

In 1997, a ToMoV-viruliferous whitefly colony was established by obtaining tomato plants infected with ToMoV from P. Stansly, University of Florida, Immokalee, FL, USA, and infesting them with whiteflies from a non-viruliferous colony. In 2001, a TYLCV-viruliferous whitefly colony was established by obtaining cuttings from field-grown tomatoes infected with TYLCV from D. Schuster, University of Florida, Bradenton, FL, USA. Rooted cuttings were planted in ~15-cm pots and infested with whiteflies from a non-viruliferous colony. After their establishment, serial transfers on dwarf cherry tomato cultivars maintained both virus colonies. The presence of the viruses was assessed by visual identification of symptom development and PCR amplification with virus-specific primers (Pico *et al.*, 1998; Sinisterra *et al.*, 1999) (Table 1).

Experimental design. Approximately 10 000 mixed-sex whiteflies were collected from laboratory colonies feeding on either ToMoV- or TYLCV-infected tomatoes. A mixed-sex population of approximately 2500 adults was immediately frozen in liquid nitrogen and stored at −80 °C and the remainder was placed on 18 cotton plants. After 2 days, whiteflies were transferred to 18 new cotton plants by severing the whitefly-infested cotton at the crown and adding the new cotton plants. Over a 24 h period, the whiteflies moved from the wilting plants to the new cotton and the severed and wilted plants were removed. At 4 days after infestation, approximately half of the viruliferous whiteflies were vacuumed from the plants with a Makita Handy Vac (model 4071D) outfitted with plastic vials screened at the bottom for collection. Representative plant samples were taken from the youngest cotton leaves on which extensive whitefly feeding had occurred. Plant material and whiteflies were immediately frozen in liquid nitrogen then stored at −80 °C until ready for analysis and new plants were introduced as before.

After a total of 7 days on cotton, the remaining whiteflies were harvested and stored at −80 °C. At both 4 and 7 days, cotton leaf samples were taken and used as controls in quantitative PCR experiments. Furthermore, the cotton plants were held for an additional 30 days and then tested for the presence of virus. At no time during this 30 day period were virus symptoms observed and, after 30 days, no virus DNA could be detected by PCR analysis (see Supplementary figure available in JGV Online). This experiment was repeated three times.

Nucleic acid extraction. Total DNA was extracted from groups of 10 whiteflies by grinding whole insects in liquid nitrogen and using the AquaPure Genomic DNA isolation kit (Bio-Rad) according to the manufacturer's instructions. DNA from 0·1 g tomato leaf tissue was extracted following the DNA extraction protocol described by Edwards *et al.* (1991). For cotton samples, 70 mg leaf tissue was processed as described by Kobayashi *et al.* (1998).

Table 1. List of RRT-PCR and RT-PCR primers used

Primer sequences for TYLCV and ToMoV were first described by Pico *et al.* (1998) and Sinisterra *et al.* (1999), respectively.

Virus/plant	Gene target	Primer sequence	Primer coordinates	Accession no.*
TYLCV	V1†	5′-CGCCCGTCTCGAAGGTTC-3′	501/518	AB110217
		5′-GCCATATACAATAACAAGGC-3′	1159/1178	
TYLCV	V1	5′-GAAGCGACCAGGCGATATAA-3′	483/502	AB110218
		5′-GGAACATCAGGGCTTCGATA-3′	655/674	
TYLCV	V2	5′-TCTGTTCACGGATTTCGTTG-3′	353/372	AB110218
		5′-GCTGTCGAAGTTCAGCCTTC-3′	533/552	
TYLCV	C3	5′-TGAGGCTGTAATGTCGTCCA-3′	1423/1442	AB110218
		5′-GCTCCTCAAGCAGAGAATGG-3′	1607/1626	
ToMoV	AV1†	5′-GCCTTCTCAAACTTGCTCATTCAAT-3′	52/76	L14460
		5′-GTTCGCAACAAACAGAGTGTAT-3′	1145/1124	
ToMoV	AV1	5′-GACGTCGGAGCTCGATTTAG-3′	2309/2338	L14460
		5′-GAGCTTCATGAAAATGGGGA-3′	2445/2464	
ToMoV	BC1	5′-CTGTACGGGTGGAGTTCGTT-3′	1520/1539	L14461
		5′-TTTCCCATGTGGATTACGGT-3′	1681/1696	
ToMoV	BV1	5′-TTTGCATACATTCGACGAGC-3′	937/956	L14461
		5′-ATCCTTCAACGTCCACCATC-3′	1072/1091	
Cotton	β-Actin	5′-GTGGCTCCAGAAGAACA-3′	412/428	AF059484
		5′-ACGACCACTGGCATATAGGG-3′	567/548	
Tomato	β-Actin	5′-GGAAAAGCTTGCCTATGTGG-3′	773/791	BT013524
		5′-CCTGCAGCTTCCATACCAAT-3′	932/948	
Tomato	Rubisco	5′-CCTGATTTGTCTGACGAGCA-3′	253/272	M15236
		5′-GCACCCAAACATAGGCAACT-3′	411/430	
Whitefly	β-Actin	5′-TCTTCCAGCCATCCTTCTTG-3′	104/123	AF071908
		5′-CGGTGATTTCCTTCTGCATT-3′	277/258	
Whitefly	18S rRNA	5′-AAACGGCTACCACATCCAAG-3′	453/472	U20401
		5′-GTCCTCGTCGCCTTGTTTAC-3′	578/597	

*GenBank accession numbers of the sequences used for primer design and from which primer coordinates were calculated.
†Primers used for testing tomato plants for the presence of TYLCV and ToMoV.

Total RNA extractions were performed using 500 mg tissue from previously frozen samples of tomato or cotton plants or approximately 2500 whiteflies. Samples were ground to a fine powder using mortar and pestle in the presence of liquid nitrogen, then processed with the RNeasy midiprep kit (Qiagen), following the manufacturer's protocol for isolation of total RNA. Trace DNA contamination was removed from total RNA preparations by a double extraction with one volume acid phenol:chloroform (5:1, pH 4·7; Ambion), followed by precipitation with two volumes 95 % ethanol and 1/10 volume 2 M sodium acetate (pH 4·0) overnight at −20 °C. RNA was pelleted by centrifugation for 5 min at 12 000 *g* and resuspended in water. RNA (10 μg) was digested with 2 U DNase I (Ambion) in a 25 μl reaction mix for 1 h at 37 °C. After a second precipitation, the sample was resuspended in water and digested with 30 U RecJ (New England Biolabs) ssDNA-specific exonuclease, 2 U DNase I, 1 × RecJ reaction buffer in a 25 μl reaction mix for 1 h at 37 °C. This sample was used directly for quantitative PCR applications.

Virus DNA and RNA quantification. A Rotor-Gene RG-3000 (Corbett Research) real-time PCR machine coupled with the DNA minor groove binding fluorescent dye SYBR Green I were used for quantitative PCR methods. Specific primers were designed to amplify segments of <200 bases from transcripts containing the following genes: ToMoV AV1, BC1 and BV1; TYLCV V1, V2 and C3; tomato β-actin; whitefly β-actin; and cotton β-actin (Table 1). AV1 and V1 encode coat proteins of ToMoV and TYLCV, respectively, BC1 and V2 encode proteins believed to be involved in cell-to-cell movement, and BV1 encodes a nuclear shuttle protein, which has no direct counterpart in the TYLCV genome, but was chosen to determine if a transcript encoding a protein with this function was expressed in the insect vector. The C3 gene, encoding a replication enhancer, was chosen to monitor complementary strand transcripts. Prior to initiation of the experiment, viral PCR amplicons were verified by sequence analysis (data not shown) and amplification of viral sequences was shown to be specific to whiteflies viruliferous for the virus being tested.

Real-time RT-PCR (RRT-PCR) was conducted using 300 ng total RNA from every sample in a 25 μl reaction mix using the Quantitect SYBR Green RRT-PCR kit (Qiagen) under recommended reaction conditions. Reverse transcription was performed for 30 min at 50 °C followed by a 15 min denaturation at 95 °C and 40 cycles of 40 s at 95 °C, 40 s at 58 °C and 40 s at 72 °C. For each sample, RRT-PCR quantification was based on relative abundance, as determined by C_t value compared with either plant or whitefly β-actin C_t. Simultaneously, the retention of plant transcripts in all ToMoV- and TYLCV-viruliferous whitefly samples was monitored using primers specific for tomato ribulose-bisphosphate carboxylase (Rubisco, 4.1.1.39) (Table 1).

In order to detect DNA contamination in the RNA samples, RRT-PCR

was conducted with 300 ng total RNA from all plant and insect samples. The Quantitect SYBR PCR kit (Qiagen) was used for RT-PCR. For detection of viral genomic sequences in total DNA samples, 300 ng total DNA was used following the above cycling profile using primer sets for the selected virus genes and β-actin. The relative titres of ToMoV and TYLCV in infected tomato and in all whitefly samples were determined using RT-PCR with primers for AV1 and V1 genes, respectively.

All real-time experiments (RRT-PCR and RT-PCR) were conducted in triplicate for each sample and melting curve and agarose gel analyses were performed to verify single product formation. Relative quantification analysis was performed using a dynamic amplification efficiency determination for each amplification run as provided in the comparative quantification function with the Rotor-Gene RG-3000 software (described in the technical bulletin entitled 'An Explanation of the Comparative Quantification Technique Used in the Rotor-Gene Analysis Software', Matthew Herrmann, Corbett Research, Mortlake, Australia). Briefly, the following exponential growth model represents the increase in fluorescence (R) during the amplification: $R_{n+1} = R_n{}^*(A)$, where n is the cycle number and A is the amplification value, a measure of reaction efficiency. The first differential was taken to remove the fluorescence background. Fluorescence increase during the exponential phase was monitored by a rearrangement of the above formula to give an observed amplification (A_n) at each point within the exponential phase of a reaction: $(A_n) = R_{n+1}/R_n$. The mean amplification over these points produced an amplification value for the sample (A_s). The mean amplification of all samples (A) was then determined and the variance was used to provide a measure of error. The amount of gene product in any given sample relative to a designated reference sample was then calculated using the formula: (A)^(control take-off point−sample take-off point). Error coefficient was determined with a 95 % confidence interval.

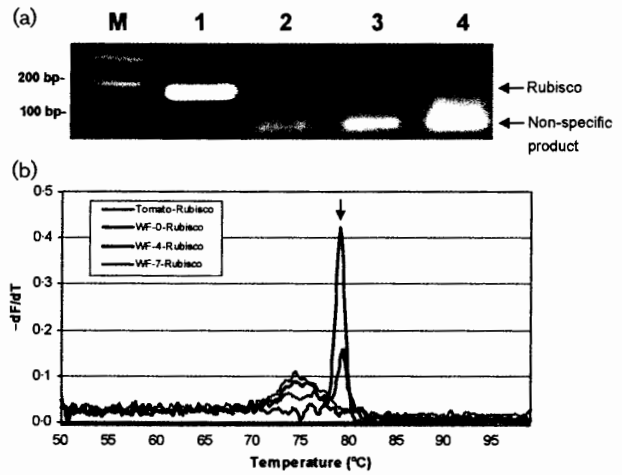


Fig. 2. Detection of tomato Rubisco transcripts in tomato leaves and ToMoV-viruliferous whiteflies by RRT-PCR. (a) Agarose gel electrophoresis (1 %) of RRT-PCR products from reactions using tomato Rubisco-specific primers (Table 1) performed on total RNA isolated from ToMoV-infected tomato plants (lane 1) and from whiteflies feeding on: ToMoV-infected tomato (lane 2), cotton for 4 days after transfer from ToMoV-infected tomato (lane 3) and cotton for 7 days after transfer from ToMoV-infected tomato (lane 4). M: 50 bp marker. (b) Melt curve analysis of RRT-PCR products showing the change in fluorescence (−dF/dT, the negative of the derivative of the fluorescence over the derivative of the temperature) plotted as a function of temperature. The arrow indicates the T_m for the amplified tomato Rubisco fragment.

RESULTS AND DISCUSSION

Clearing the insect alimentary tract of ingested RNA transcripts

Prior to virus transcript quantification, a method was needed to distinguish between transient ingested viral transcripts in the gut and virus transcripts that represent a pool of more stably acquired or newly synthesized RNA molecules. Using RRT-PCR, ingested tomato Rubisco transcripts (abundant in leaf tissue) could be detected for at least 3 days in whiteflies removed from tomato and fed on an artificial sucrose diet (data not shown); however, these transcripts were not detected in whiteflies that had been removed from tomato and fed on cotton for at least 4 days (Fig. 2). Fig. 2 shows this result only for ToMoV experiments; however, similar results were observed with TYLCV-infected and non-infected plants, indicating that there was no virus influence on stability of plant RNA within the whitefly. Non-specific product formation occurred when template concentration was low, but no specific product was visible in the melt curve analysis of RRT-PCR results from any whiteflies fed on cotton (Fig. 2a and b). Non-specific product formation was periodically observed for some primer pairs when template was not available. Quantitative experiments were all performed with reactions where sufficient template was provided to minimize non-specific product formation (as in Fig. 2a, lane 1). For subsequent experiments, cotton feeding was used to clear ingested virus RNA within the gut, thus allowing measurement of only ingested RNA entering protected environments or newly synthesized transcripts.

Differential abundance of TYLCV and ToMoV transcripts within the whitefly

Mixed-age adult whiteflies feeding on virus-infected tomato were transferred to cotton and allowed to feed for up to 7 days, with populations sampled after 4 and 7 days for virus transcript quantification. ToMoV AV1 and BV1 (A and B component virus strand genes, respectively) transcripts were detected only in the ToMoV-infected tomato and whiteflies collected immediately from those plants (Fig. 3a, b, c, d). The BC1 (B component complementary strand gene) transcripts were detected in ToMoV-infected tomato, but not in any of the whitefly samples (Fig. 3e, f). In these experiments, the detection and quantification of whitefly β-actin also indicated that the inability to detect ToMoV gene transcripts was not due to reaction failure or to major differences in total RNA amounts used. Gel electrophoresis was also performed on each reaction in Fig. 3 to verify product formation and ToMoV transcripts or DNA were not detected in cotton (Supplementary figure in JGV Online).

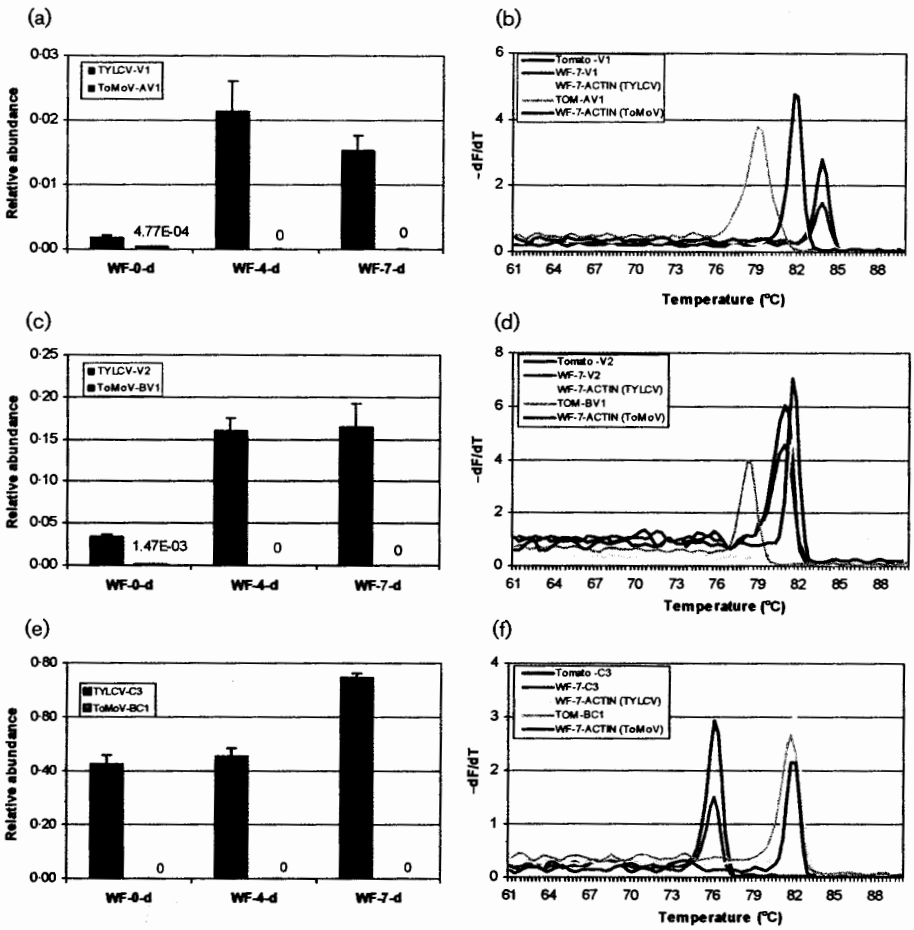
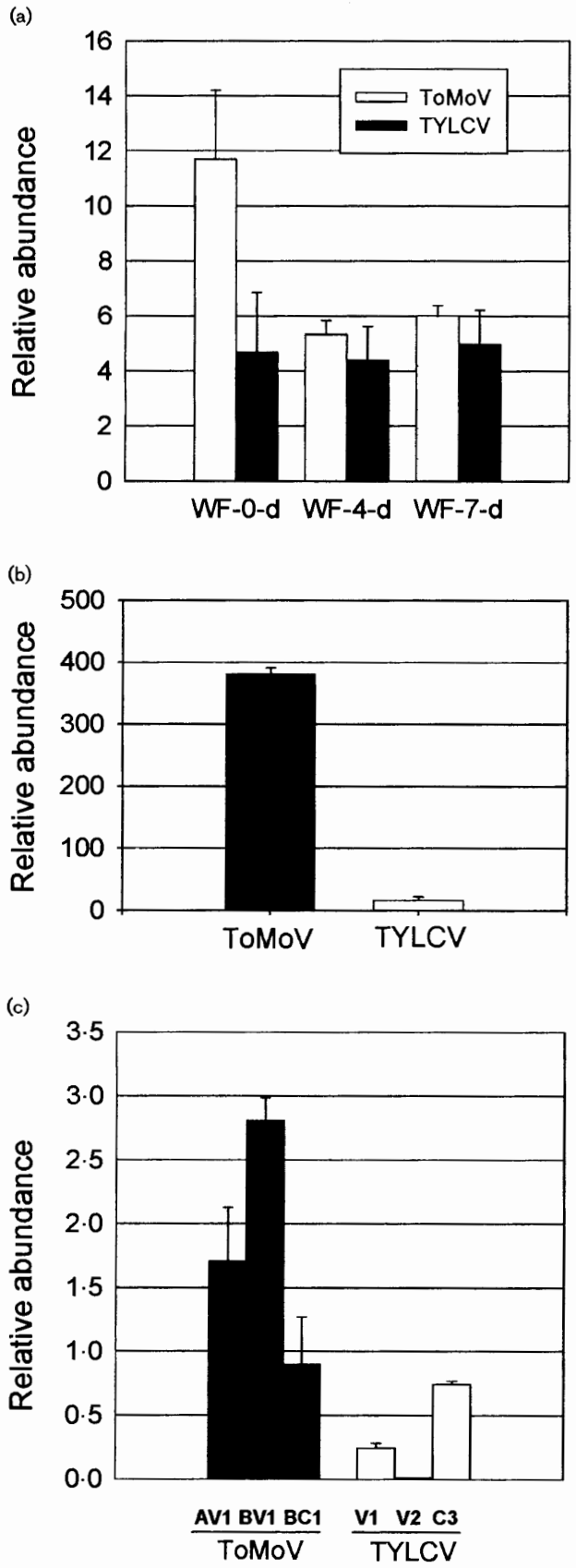


Fig. 3. RRT-PCR quantification of ToMoV and TYLCV gene transcripts in infected tomato and viruliferous whiteflies. The RRT-PCR quantification method relying on dynamic calculation of amplification efficiency (see Methods) and relative quantification compared with β-actin was used for viral transcript quantification within total RNA samples. Sequences of transcript-specific primers used are listed in Table 1. (a), (c) and (e) show the relative transcript quantification value plotted as a function of whitefly treatment: WF-0-d, whiteflies removed directly from tomato; WF-4-d, whiteflies sampled from cotton 4 days after transfer from virus-infected tomato; WF-7-d, whiteflies sampled from cotton 7 days after transfer from virus-infected tomato. Whitefly populations were viruliferous for TYLCV or ToMoV as indicated in the figure, along with the specific transcript quantified. (b), (d) and (f) show melt curve analysis of RRT-PCR products produced from either tomato or whitefly total RNA plotted as the change in fluorescence (−dF/dT, the negative of the derivative of the fluorescence over the derivative of the temperature) as a function of temperature. Template/primer combinations were as follows: TYLCV-infected tomato total RNA with TYLCV V1, V2 or C3 (Tomato-V1, -V2 and -C3, respectively); ToMoV-infected tomato total RNA with ToMoV AV1, BV1 or BC1 (TOM-AV1, -BV1 and -BC1, respectively); whiteflies sampled from cotton 7 days after transfer from TYLCV- or ToMoV-infected tomato with primers for either the viral transcripts or whitefly β-actin (WF-7-V1, -AV1, -V2, -BV1, -C3, -BC1, -ACTIN). Quantitative data represent combined results obtained from three complete biological replicates. Agarose gel electrophoresis of all reaction products and negative controls for each reaction are shown in Supplementary figure (available in JGV Online).

In contrast to the results for ToMoV, transcripts of TYLCV V1, V2 (virus strand genes) and C3 (complementary strand gene) were detected in TYLCV-infected plants and all the whitefly samples. In fact, the relative abundance of V1, V2 and C3 gene transcripts was significantly higher in whiteflies after 4 and 7 days of feeding on cotton than in whiteflies collected from TYLCV-infected tomato prior to the transfer to cotton (Fig. 3a, c, e). Melt curves show single products produced in amplifications of both viral and β-actin transcripts (Fig. 3b, d, f). There was no product amplification in reactions that did not have the reverse transcriptase step (Supplementary figure in JGV Online).

The apparent increase of TYLCV transcripts is based on relative abundance of the viral transcripts compared with the whitefly β-actin transcript and therefore could be due to an increase in total number of viral transcripts or to a decline in the abundance of the whitefly β-actin transcript, possibly



a result of whitefly colony ageing or changing the whitefly host plant and thus nutritional status. Relative determination of whitefly β-actin abundance to whitefly 18S rRNA indicated that, after 4 days of feeding on cotton, there was no change in whitefly β-actin abundance relative to the 18S rRNA, but there was still a significant increase in virus transcripts relative to this rRNA (data not shown). The increase in viral transcripts relative to either whitefly 18S rRNA or β-actin was surprising, since it was previously shown that begomovirus DNA reached a steady-state level within the whitefly after approximately 10 h of feeding on a single virus-infected host (Czosnek *et al.*, 2001). Our results, indicating an increase in virus transcript abundance after transferring whiteflies to a new host, can only be explained by TYLCV transcriptional activity within the whitefly. This result may suggest a dynamic control over TYLCV transcription or at least changes in viral transcript stability in response to changes in whitefly physiology (lack of ingested virus, whitefly ageing or altered nutritional status).

Comparison of virus genome titre and viral transcript abundance in viruliferous whiteflies and infected tomato

Virus genomic DNA (ssDNA viral genome and replicative dsDNA) abundance was determined using RT-PCR and primers for V1 (TYLCV) and AV1 (ToMoV) DNA detection. The ToMoV titre in whiteflies sampled directly from infected tomato was about three times higher than the virus titre in TYLCV-viruliferous whiteflies similarly sampled and, after 4 days of feeding on cotton, a significant decrease in ToMoV titre to the level equal to the TYLCV titre was observed (Fig. 4a). Conversely, TYLCV titre in viruliferous whiteflies remained constant even after 7 days of feeding on cotton plants (Fig. 4a). The rapid decline in ToMoV DNA abundance in whiteflies transferred to cotton compared

Fig. 4. Titre of ToMoV and TYLCV DNAs in infected tomato and whiteflies feeding on infected tomato and virus transcript abundance in infected tomato plants. The RT-PCR quantification method relying on dynamic calculation of amplification efficiency (see Methods) and relative quantification compared with whitefly or tomato β-actin was used for viral DNA quantification in total DNA samples using the AV1-specific primers for ToMoV and the V1-specific primers for TYLCV. Primer sequences are listed in Table 1. (a) Relative DNA quantification value plotted as a function of whitefly treatment: WF-0-d, whiteflies removed directly from tomato; WF-4-d, whiteflies sampled from cotton 4 days after transfer from virus-infected tomato; WF-7-d, whiteflies sampled from cotton 7 days after transfer from virus-infected tomato. Whitefly populations were viruliferous for either TYLCV or ToMoV as indicated in the figure. (b) Abundance of viral DNA in either ToMoV- or TYLCV-infected tomato relative to tomato β-actin. (c) RRT-PCR quantification of the relative abundance of selected gene transcripts of TYLCV (V1, V2, C3) and ToMoV (AV1, BV1, BC1) in infected tomato used to rear viruliferous whiteflies. RRT-PCR quantification was performed as described in (a).

with the lack of a similar drop in TYLCV suggests a higher ToMoV DNA load present in the gut contents that was cleared during feeding on cotton. Since whiteflies are strict phloem-feeding insects, a higher abundance of ToMoV DNA compared with TYLCV DNA in the phloem would account for this difference and, in support of this hypothesis, quantification of viral DNA titre in tomato indicated a ToMoV DNA titre more than 10 times higher than the TYLCV DNA titre (Fig. 4b) in total leaf tissue. Furthermore, the finding that TYLCV transcripts are less abundant in leaf tissue than ToMoV transcripts (at least in comparisons of V1 and V2 to AV1 and BV1) (Fig. 4c) is also supportive evidence showing that the stable detection of TYLCV transcripts in the whitefly is not the result of differences in bulk loading of these transcripts from feeding (i.e. whiteflies are not incorporating more TYLCV transcript from their diet than they are ToMoV transcripts). One caveat to these observations is that our quantification of TYLCV and ToMoV DNA and RNA transcripts was performed on whole leaf samples and tissue specificity of TYLCV is still uncertain in view of published observations of phloem-specific localization (Morilla *et al.*, 2004) and localization to both mesophyll and phloem (Michelson *et al.*, 1997). Tissue specificity of ToMoV is also uncertain. Therefore, it is not clear if the relative abundance relationship between the two viruses as measured in whole leaves would be maintained in phloem to phloem comparisons (the feeding location of the whitefly). Since the relative distribution of virus DNA and transcripts within different leaf tissues is uncertain, differences in whitefly feeding uptake as opposed to tissue distribution of virus cannot accurately be assessed.

Conclusion

Our data show that, in a whitefly population feeding on tomato infected with an Old World begomovirus, TYLCV, and transferred to cotton (not a TYLCV host), virus transcripts were stimulated to accumulate in the whitefly, whereas the viral DNA components (ssDNA genomic and/or dsDNA replicative form) remained constant. However, in response to the same host transfer, transcripts in the whitefly of the New World begomovirus ToMoV declined to undetectable levels and the viral DNA abundance dropped significantly. Accumulation of TYLCV gene transcripts in the whitefly was suggested by Czosnek *et al.* (2001) using non-quantitative RT-PCR methods to show the presence of TYLCV V1 gene transcripts 3 h after a 1 h acquisition access period. However, our work shows that when whiteflies feeding on TYLCV-infected tomato were transferred to a host that was immune to TYLCV, all transcripts (V1, V2 and C3) increased in abundance. This increase suggested *de novo* transcript synthesis and also indicated that the accumulation of TYLCV transcripts was not held at a constant level within the whitefly.

Previous reports have shown that TYLCV appears to reduce whitefly fitness, whereas ToMoV does not. It has been speculated that this may be the result of the ability of TYLCV to replicate in the whitefly (Czosnek *et al.*, 2001). Whether this occurs or not, our data show interesting differences in transcriptional activity of the two viruses that may be related to the reported influences of these viruses on whitefly biology. Czosnek *et al.* (2001) have further hypothesized that begomoviruses may represent a genus with species that are undergoing an evolutionary divergence in the mechanisms by which they interact with their insect vectors.

In that context, it is interesting to speculate that the differences in accumulation of genomes and transcripts in the plant and the insect may represent two different strategies for maintaining a virus titre within the vector sufficient to assure transmission to other host plant species. ToMoV, a virus that does not replicate within the whitefly, accumulates to a high titre in the plant, allowing rapid saturation of the insect with virus particles and rapid replenishment of this pool as the whitefly feeds. TYLCV, a virus displaying genetic activity (transcription and, by inference, replication) in the whitefly, does not require production of such high virus titres in the plant, as it would be maintained in the insect vector by virus replication. This implies that the ability to replicate in the insect may obviate the insect's need to constantly take in high levels of virus to assure future virus transmission.

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